Brain Penetrant CDK4/6 Inhibitor PRT3645 Demonstrates Anti-tumor Activity and Enhances Survival in Glioblastoma and Breast Cancer

Brain Metastasis Models

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Background

- Cell-cycle deregulation is hallmark of cancer and cycle-dependent kinase (CDK) inhibitors specifically inhibit CDKs at different cell-cycle phases to halt the progress of the cell cycle.
- CDK4/6 inhibitors are the first and only class of highly specific, CDK inhibitors approved for cancer treatment indications.
- CDK4/6 inhibitors have transformed the treatment paradigm of estrogen receptor-positive (ER+), human epidermal growth factor receptor 2-overexpressing (HER2+) breast cancer, with three CDK4/6 inhibitors currently approved in the US.

Objective

To profile the biochemical pharmacological activity of PRT3645, a brain-penetrant CDK4/6 inhibitor, both in vitro and in vivo in various cancers, including GBM and BCBM, as a single agent as well as in combination with existing cancer treatments.

Key Findings

- PRT3645 inhibits cellular phosphorylation of metastasates (Ki) protein with low nanomolar activity.
- PRT3645 treatment resulted in inhibition of cell proliferation of various tumor types with most cell lines showing an IC50 of <100 nM.
- PRT3645 was well-tolerated and highly efficacious in a xenograft model of breast cancer as well as ER+ and HER2+ models of glioblastoma (GBM) and breast cancer brain metastases (BCBM).

Results

Figure 1: PRT3645 Is a Potential and Selective CDK4/6 Inhibitor With High Brain Penetration Compared With Approved CDK4/6 Inhibitors

- PRT3645 is a potent and selective CDK4/6 inhibitor with high brain penetration compared with approved CDK4/6 inhibitors. PRT3645 is an orally bioavailable, brain penetrant, and potent CDK4/6 inhibitor with >1000-fold selectivity over CDKs 1-7, 9, and 12.

Methods

- CyclinD-CDK4/6-Rb-E2F Pathway
  - Breast cancer and glioblastoma cells were seeded at 100,000 cells/well in 6-well plates, incubated for 24 hours with PRT3645. Actin was used as a control for equal loading and stability of protein levels in the cell line.

Conclusions

- PRT3645 is an orally bioavailable, brain penetrant, and potent CDK4/6 inhibitor with >1000-fold selectivity over ER+ breast cancer cells and GBM cells.
- Across various tumor types, PRT3645 reduced cell viability with the majority of cell lines showing an IC50 of <100 nM.
- PRT3645 is highly effective in reducing cell viability of various tumor types in an in vitro panel (Cell Proliferation) Cell Proliferation Assay.
- Cells were seeded in a 24-well plate, and PRT3645 was serially diluted 3.125% from the highest tested concentration of 20 nM and assayed (CisScreen™ Cell proliferation assay) over two concentrations with a maximum assay concentration of 0.1% DMSO. Automated fluorescence microscopy was carried out using Enhanced Luminol Chemiluminescence (ELC) and brightness corrected to fluorescent intensity (FI) with high content imaging, and images were analyzed with Metaphosphor® 5.3.0.1 software.
- Figure 2: PRT3645 Inhibits Cellular Phosphorylation of Rb With Low Nanomolar Activity
  - Cells were treated in a concentration-dependent manner, and western blotting analysis was performed for pRb 807/811 and pRb 708 in cells treated for 24 hours with PRT3645. Actin was used as a control for equal loading and stability of protein levels in the cell line.
  - Downregulation of pRb, reduction in S-phase of the cell cycle, and potent inhibition of cell proliferation were observed with PRT3645 treatment. Figure 3: PRT3645 Is Highly Effective in Reducing Tumor pRb in a Single-Dose PK/PD Study in a U-87 MG Subcutaneous GBM Model
  - A U-87 MG subcutaneous GBM xenograft model was established by injecting cancer cells (3.0×10⁶ cells/mouse, with 50% Matrigel™) subcutaneously in the right flank of female nude mice. Animals were treated with an escalating dose of PRT3645, and treated tumors were examined at the 12th hour post-treatment, SD, single dose, PK/PD pharmacokinetics.

- CyclinD-CDK4/6-Rb-E2F Pathway
  - Breast cancer and glioblastoma cells were seeded at a 96-well plate, and PRT3645 was dispersed using a Tecan 760 μL liquid handling robot. After a 24-hour incubation, cell viability was measured using a Cell counting kit-8 (CCK-8) colorimetric assay that measures activity of dehydrogenases in cells which is directly proportional to the number of living cells. IC50 values were calculated by using GraphPad Prism 5.1.1 software.

- Figure 4: PRT3645-Treated Breast Cancer Cells Show Cell Cycle Inhibition With A Strong Reduction In the S-Phase
  - Cells were treated with PRT3645 in a concentration-dependent manner for 24 hours. Cell cycle phases (G0, G1, and G2/M) were measured by Propidium iodide (PI) and 7-aminoactinomycin D DNA dye (DAPI) staining. Total flux data were evaluated using flow cytometry, and the data were processed using FlowJo software. (A) Breast cancer cells. (B) Glioblastoma cells.